LEGENDS FOR SUPPLEMENTARY FIGURES

FIGURE S1

Further mass spectrometric analysis of the Myddosome.

Upper/Middle Panels: Confirmation of 7:4 and 8:4 MyD88:IRAK4 stoichiometries by tandem mass spectrometry (MS/MS). Selected parent ions are isolated in the instrument's quadrupole mass analyzer, prior to activation by collisions with argon atoms. Gas-phase collision-induced activation of a protein complex typically leads to ejection of a single, highly charged (i.e. low m/z) subunit from a residual "stripped" complex retaining relatively low charge (i.e. high m/z) (1). Upper panel = MS/MS of the 7:4 complex +26 ion. The spectrum shows dissociation via two pathways giving IRAK4 and MyD88 subunits respectively (observed masses 12,461 Da and 16,570 Da respectively), and corresponding "stripped" complexes of stoichiometry 7:3 (observed mass = 153,285 Da, expected mass = 153,272 Da) and 6:4 (observed = 149,192 Da, expected = 149,166 Da). Lower panel: similar analysis of the 8:4 complex +29 ions, which dissociates to give IRAK4 and MyD88 monomers, plus stripped complexes of stoichiometry 8:3 (observed = 169,857 Da, 169,831 Da) and 7:4 (observed = 165,748 Da, 165,725 Da). Lower Panel: Mass spectrum showing the high m/z "stripped complex" ion series generated by gas-phase activation of the full range of complex ion series (i.e. without pre-selection in the mass spectrometer's quadrupole). The major ion series correspond to the expected gas-phase dissociation products from 7:4 and 8:4 MyD88:IRAK4 parent complexes. Cyan stars = MyD88:IRAK4 7:3 stoichiometry (arising from gas-phase loss of an IRAK4 subunit from a 7:4 parent ion), observed mass 153,301 ± 27 Da, expected mass = 153,272 Da. Yellow triangles = 6:4, (loss of MyD88 from a 7:4 parent), observed = 149,187 ± 24 Da, expected = 149,166 Da. Blue ellipses = 8:3 (loss of IRAK4 from a 8:4 parent), observed = 169,855 ± 50 Da, expected = 169,831 Da. Green circles = 7:4 (loss of MyD88 from a 8:4 parent), observed = 165,760 ± 18 Da, expected = 165,725 Da. Weaker ion series (not labelled) include ions corresponding to the stoichiometries 7:2 (observed = 140,840 ± 7 Da, expected = 140,819 Da) and 6:3 (observed = 136,772 ± 50 Da, expected = 136,713 Da), implying the presence of a minor 7:3 component in the unresolved peaks shown in Figure 3B.

FIGURE S2.

Detailed view of model 1 and 2 image reconstruction.

Top panel: Reconstruction of MyD88-IRAK4 DD, model 1. (a) Representative view of the electron micrograph for MyD88-IRAK4 DD complex. The images were taken at 40,000X magnification. The scale bar corresponds to 34 nm. These micrographs were used to select particles that were used to generate both model 1 and model 2. (b) Class averages of the particles for the structure in model 1. (c) Fourier shell correlation showing convergence at about 28 Å resolution. The convergence was also confirmed by comparing the projections of the model with corresponding class averages of the particles (data not presented). (d) Asymmetric triangle showing the sampling of the particles in the reconstruction process. (e) Different views of the final model. Approximate dimensions of the model are shown in the upper left particle. Also shown is the cut-view of the model to show the presence of a cavity in the model.

Bottom panel Reconstruction of MyD88-IRAK4 DD, model 2 (a) Class averages of the particles used to generate model 2. (b) Asymmetric triangle showing the sampling of the particles in the reconstruction process. (c) Fourier shell correlation of the reconstruction showing the final model converged at approximately 29 Å. The convergence was also tested by comparing the projections of the model with corresponding class averages. (d) Different view of the final model. Approximate dimensions of the model are shown in the upper left particle. Cut-views are shown next to each of the model to show the presence of the cavity inside the model

1.   Benesch JL, et al. 2006 Tandem mass spectrometry reveals the quaternary organization of macromolecular assemblies. *Chem Biol* 13:597-605.
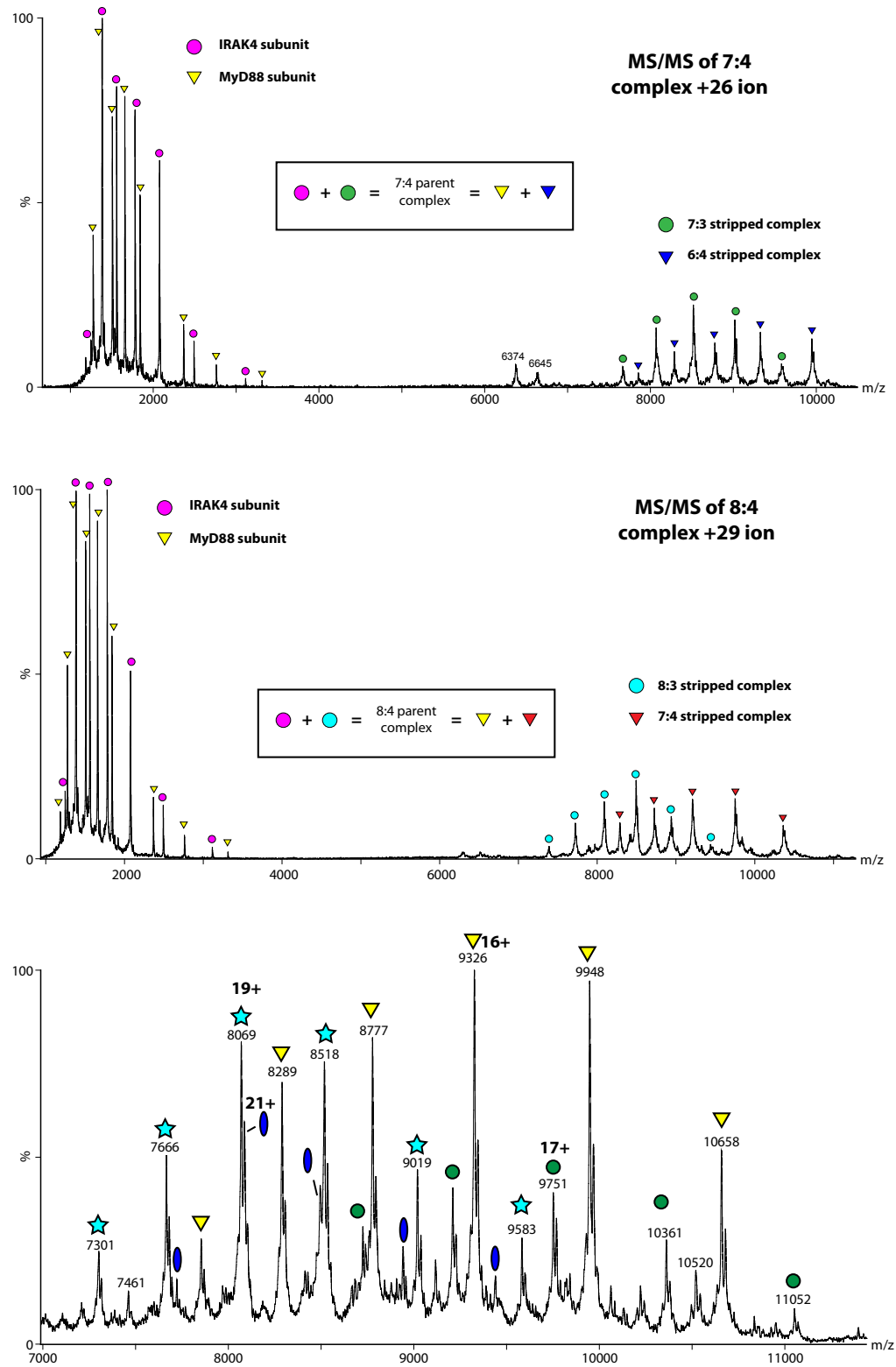
FIGURE S1

FIGURE S2

34 nm

Resolution ~ 28 Å

73 Å

88 Å

Cut view

Supplemental Fig.1 (Model 1)

62 Å

80 Å

Cut view

Supplemental Fig.1 (Model 2)

Resolution ~ 29 Å

4